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Review Article

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Abstract

Vision loss, among the most feared complications of diabetes, is primarily caused by diabetic retinopathy, a disease that manifests in well-recognized, characteristic microvascular lesions. The reasons for retinal susceptibility to damage in diabetes are unclear, especially considering that microvascular networks are found in all tissues. However, the unique metabolic demands of retinal neurons could account for their vulnerability in diabetes. Photoreceptors are the first neurons in the visual circuit and are also the most energy-demanding cells of the retina. Here, we review experimental and clinical evidence linking photoreceptors to the development of diabetic retinopathy. We then describe the influence of retinal illumination on photoreceptor metabolism, effects of light modulation on the severity of diabetic retinopathy, and recent clinical trials testing the treatment of diabetic retinopathy with interventions that impact photoreceptor metabolism. Finally, we introduce several possible mechanisms that could link photoreceptor responses to light and the development of retinal vascular disease in diabetes. Collectively, these concepts form the basis for a growing body of investigative efforts aimed at developing novel pharmacologic and nonpharmacologic tools that target photoreceptor physiology to treat a very common cause of blindness across the world.

Introduction

Diabetic retinopathy (DR) is a leading cause of acquired blindness, but its pathogenesis requires clarification. Although it ultimately manifests as a characteristic microvasculopathy, DR is associated with abnormalities of many retinal cell types preceding the onset of microvascular deterioration (Honasoge et al., 2019). In fact, the photoreceptor—the first cell of the visual circuit—is an important contributor to retinal vascular disease in diabetes (Liu et al., 2016). The retina's remarkable metabolic signature could underlie its exceptional vulnerability to damage from the metabolic perturbation caused by diabetes (Antonetti et al., 2006), and photoreceptors are the most metabolically demanding cells in the retina (Wong-Riley, 2010) making them a prime target of early disease. Furthermore, since illumination of the retina determines the energy expenditure of photoreceptors, light exposure itself may influence the pathogenesis and course of retinal diseases, such as DR (Arden et al., 2005). Therefore, light-dependent modulation of photoreceptor physiology in early diabetes has been a considerable focus of study. In this review, we will briefly discuss the formative evidence implicating photoreceptor physiology in the development of DR. Next, we will address how these early observations combined with light-dependent effects on photoreceptor metabolism gave rise to the hypothesis that light exposure could modulate the pathogenesis of this disease. Finally, we will describe the studies designed to test this hypothesis and introduce a variety of mechanistic possibilities arising from their findings.

Photoreceptor dysfunction in diabetes

Clinical evidence

The strongest evidence for photoreceptor involvement in DR comes from clinical observations. First, elevated levels of a photoreceptor-secreted protein—retinol-binding protein 3 (RBP3)—are linked to protection from DR, as reported by investigators at the Joslin Diabetes Center who participated in the MEDALIST trial, a study of patients with diabetes who survived with 50 years or more of disease. The protective effects of RBP3 were also seen in overexpression studies using a common mouse model of diabetes. Although RBP3 is an integral protein in the retinoid cycle, its mechanism of action in DR could be related to direct binding and negative regulation of GLUT1 transporter—the major glucose transporter in the retina (Yokomizo et al., 2019).

Second, patients with diabetes show electrophysiological signs of photoreceptor dysfunction. In a small but seminal electroretinography study by Holopigian and colleagues, diabetes was associated with reduced photoreceptor a-wave sensitivity, consistent with altered photoreceptor

transduction (Holopigian et al., 1997). More recently, McAnany and Park intricately dissected temporal features of the light-adapted flicker electroretinogram to provide additional evidence that diabetes likely impacts photoreceptor physiology (McAnany & Park, 2019). In addition, Holopigian and colleagues also identified diabetes-associated abnormalities of b-wave amplitude and implicit time, primarily generated by ON-bipolar cells, which may represent the effects of photoreceptor dysfunction on downstream signaling at bipolar cells (Holopigian et al., 1997). Using full-field electroretinography, Bresnick and colleagues demonstrated that a-wave implicit times were significantly delayed in 72 patients with diabetes compared to 29 healthy controls (Bresnick & Palta, 1987). Thus, patients with DR present with electrophysiological deficits in photoreceptor responses at both the receptoral and postreceptoral levels. Further evidence for abnormalities of outer retinal function in patients with diabetes comes from studies of photostress recovery, in which diabetes is associated with slower rates of photopigment regeneration after bleaching (Elsner et al., 1987). Whether these electrophysiological changes represent primary insults to the photoreceptor due to diabetes or whether they represent a consequence of early capillary damage remains unclear. This issue is made more complex by recent findings using high-resolution optical coherence tomography and optical coherence tomography angiography in which diabetes is associated with deep capillary plexus abnormalities and outer retinal structural changes, even in the absence of macular edema (Scarinci et al., 2015; 2016).

Finally, patients with coexisting diabetes and outer retinal degenerative disorders such as retinitis pigmentosa (RP) tend to be spared from severe DR. For example, Sternberg and colleagues noted a negative correlation between RP and the severity of DR (Sternberg et al., 1984). The late electrophysiologist Dr. Geoffrey Arden reported in a survey of 55 patients with RP and diabetes (with mean duration of diabetes of ~40 years) that none had clinical DR, although many had nonretinal microvascular complications of diabetes (Arden, 2001). These findings formed the basis for Arden's hypothesis that the absence of metabolically demanding photoreceptors in a degenerated retina may ease the strain on a microvascular network compromised by diabetes (Arden et al., 2005). It is important to note that all of these observations are limited by sample size, due to the rare co-occurrence of RP and diabetes and by the observational nature of the studies.

Experimental evidence

These clinical observations gave rise to the notion that photoreceptor loss in RP may ameliorate the microvasculature deterioration characteristic of DR by counterbalancing diabetes-induced retinal hypoxia. To test this hypothesis, de Gooyer and collaborators compared the severity of DR between *rhodopsin*$^{-/-}$ or wild-type controls 5 months following streptozotocin (STZ) exposure, to model type 1 diabetes. Whereas STZ-treated wild-type mice showed characteristic features of DR, including reduced retinal microvascular density and elevated inner retinal hypoxia, STZ-treated *rhodopsin*$^{-/-}$ mice did not. Moreover, STZ-associated upregulation of vascular endothelial growth factor-A (VEGF-A), a pivotal disease-promoting factor regulated by hypoxia, was attenuated in *rhodopsin*$^{-/-}$ mice. Consistent with these findings, *rhodopsin*$^{-/-}$ or P23H knock-in transgenics at 5 months and 8 months post STZ injection, respectively, showed decreased capillary atrophy compared to wild-type controls (de Gooyer et al., 2006*a*; Sakami et al., 2011). Interestingly, in both strains of mice, retinal capillary degeneration occurred during the period when photoreceptors were still present but was reduced once photoreceptor degeneration occurred. These observations suggest that primary photoreceptor diseases significantly influence microvasculature health. Although the precise molecular abnormalities linking the two remain unknown, these experimental findings appear consistent with the notion that preserving normal photoreceptor physiology could ameliorate microvasculature damage in diseases such as diabetes (Liu et al., 2016).

The results of the above studies suggest that oxygen consumption by photoreceptors in diabetes could cause pathologic inner retinal hypoxia, thereby triggering hypoxia-linked gene expression (de Gooyer et al., 2006*b*). An attractive feature of this model is that it could also explain the beneficial effects of panretinal photocoagulation (PRP) on stemming advanced features of DR (Investigators, 1978) in that laser ablation of photoreceptors could cause similar reduction of oxygen requirements as RP (Arden et al., 2005; Graham et al., 2006) (Fig. 1).

Rather than correcting hypoxia, loss of photoreceptors could improve oxidative stress due to diabetes. Retinal oxidative stress develops in diabetes due to an imbalance between the production of reactive oxygen species (ROS) and antioxidant enzymes (Kern, 2017). Though retinal ROS, such as superoxide, accumulates in multiple retinal layers in diabetes, photoreceptors are the major source of these molecules as assessed using histochemical and bioluminescence methods. *Rhodopsin*$^{-/-}$ mice have diminished diabetes-induced oxidative stress compared to wild-type controls, and this finding correlates with reduced induction of inflammatory molecules such as inducible nitric oxide synthase (iNOS) and intercellular adhesion molecule-1 (ICAM-1). Collectively, this evidence suggests that photoreceptors are major contributors to diabetes-induced retinal superoxide and inflammation (Du et al., 2013). However, the specific molecular abnormalities governing diabetes-induced activation of superoxide generation are unclear. Furthermore, more work is needed to identify the intermediate steps bridging photoreceptor oxidative stress to retinal vascular pathology in diabetes.

Energy demands of the photoreceptor in light- and dark-adapted conditions

As detailed above, although dysregulated photoreceptor physiology in diabetes is linked to the development of early retinopathy, the relevant mechanisms are unclear. Here, we provide a brief overview of the major function of photoreceptor physiology, namely the transduction of light energy into chemical signals and the energy costs of this process.

Phototransduction and the visual cycle

Mammalian photoreceptors have the remarkable ability to detect and reliably transduce light over a wide range of light intensities into an electrical response that can be relayed to the rest of the visual system (Arshavsky & Burns, 2012). Rod photoreceptors comprise nearly 95% of all photoreceptors and mediate dim light (scotopic) vision. The remaining 5% are cone photoreceptors concentrated in the central retina and provide high visual acuity and color vision under bright light (photopic) conditions (Curcio et al., 1990). Despite their important functional differences, these two types of photoreceptors share the same principles of phototransduction, the cellular mechanism of light detection (Kefalov, 2012).

Photoreceptors are characterized by their specialized outer segments containing membranous discs densely occupied by phototransduction-related signaling proteins and visual pigments.

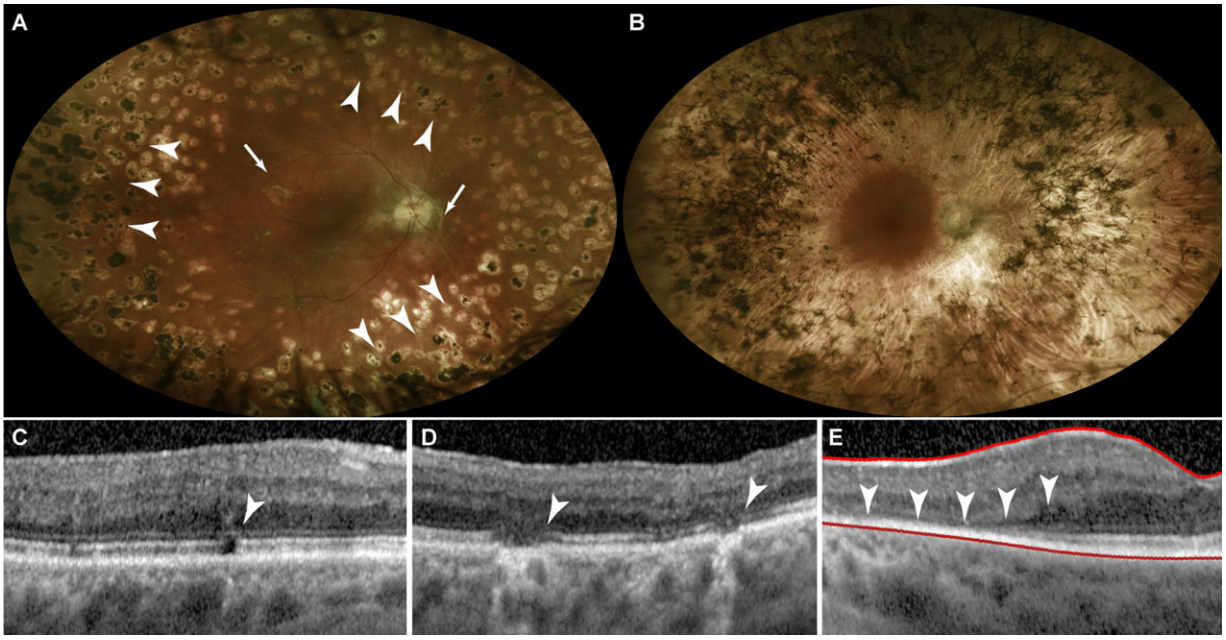

Fig. 1. Protective effects of outer retinal loss due to laser or inherited degenerations on diabetic retinopathy. **(A)** Wide-field scanning laser ophthalmoscopic image of a patient with a history of proliferative diabetic retinopathy, treated with panretinal photocoagulation. Seen are laser scars scattered in the retinal periphery (arrowheads) and signs of regression of fibrovascular disease (arrows). **(B)** Wide-field image from a patient with retinitis pigmentosa and concomitant type-1 diabetes, showing profound peripheral outer retinal atrophy and a notable absence of diabetic retinopathy. **(C)** Optical coherence tomogram showing a macular laser photocoagulation scar in the outer retina (arrowhead) in a patient with diabetes. **(D)** Outer retinal scars from peripheral laser photocoagulation in a patient with proliferative diabetic retinopathy (arrowheads). **(E)** Outer retinal loss in a patient with retinitis pigmentosa (arrowheads).

As illustrated in Fig. 2A, the visual pigment is formed by the covalent association of the G-protein-coupled receptor opsin with the light-sensing visual chromophore 11-*cis*-retinal (11-*cis*-RAL), obtained from Vitamin A (all-*trans*-ROL) via a process known as the "visual cycle" (Saari, 2000; Hargrave, 2001). Phototransduction begins when photon absorption activates visual pigment via isomerization of 11-*cis*-RAL to all-*trans*-RAL. This initiates a G-protein signaling cascade that culminates in cyclic guanosine monophosphate (cGMP) breakdown and consequent closure of cGMP-sensitive channels (Arshavsky & Burns, 2012). Reduction in sodium ion (Na^+) and calcium ion (Ca^{2+}) influx through cGMP-sensitive channels leads to photoreceptor hyperpolarization. The associated reduction of glutamate release at the synaptic terminal signals the quantity of photon absorption to the rest of the visual system (Arshavsky et al., 2002). The photoresponse persists until each phototransduction protein becomes deactivated through the action of one or more regulatory enzymes (Mendez et al., 2000; Stephen et al., 2008; Anderson et al., 2009).

Comparison of energy requirements in darkness and in light

In light-adapted conditions, photoreceptors are actively engaged in the phototransduction cascade and visual cycle (Calvert et al., 2006). In contrast, during strict dark adaptation, these processes primarily become inactive, allowing cGMP to accumulate within photoreceptors (Fain et al., 1996). Consequent opening of cGMP-sensitive channels allows influx of Na^+ and Ca^{2+} into the outer segment, which leads to membrane depolarization. Excess Na^+ and Ca^{2+} must then be extruded by Na^+/K^+ ATPase and Ca^{2+} ATPase pumps in the inner segment (Hagins et al., 1970).

As illustrated in Fig. 2B, significantly more energy is required to maintain this electrochemical gradient or "dark current" in comparison to any light-dependent photoreceptor activities, including phototransduction. In fact, energy requirement calculations show a 350% increase in energy consumption in darkness. Specifically, estimated ATP expenditure in a mouse rod photoreceptor amounts to a total of 9×10^7 ATP/s in darkness compared to only 2×10^7 ATP/s in bright illumination (Okawa et al., 2008). This disparity primarily arises from ATP-dependent ion pumping to maintain the dark current. Na^+/K^+ ATPase utilizes about 50% of total energy requirements in darkness (5.7×10^7 ATP/s), of which 70% is directed toward maintenance of the dark current. In contrast, Na^+/K^+ ATPase only requires 15% of energy in light, 0% of which is utilized for dark current. The majority of the remaining energy expenditure in darkness is attributed to Ca^{2+} ATPase activity (3×10^7 ATP/s). Although photoreceptors release more glutamate in the dark due to depolarization, glutamate synthesis and recycling only consume less than 4×10^6 ATP/s (Wong-Riley, 2010).

In bright illumination, phototransduction involves multiple steps utilizing guanosine triphosphate (GTP) and ATP; however, these requirements are minimal relative to those in darkness. GTPase activity associated with transducin utilizes less than 3×10^6 ATP/s in rods and cGMP turnover spends about 2×10^6 ATP/s. Likewise, there is negligible expenditure on the regulatory enzymes that terminate the photoresponse. Ca^{2+} channels also consume relatively little energy in bright illumination (Wong-Riley, 2010). Thus, it becomes readily apparent how total energy consumption in bright light is less than a quarter of that in the dark.

Retinal energy supply and metabolic flux

Photoreceptors are known to have extraordinary energy demands and a high density of mitochondria; however, their fuel sources are not yet clearly delineated. Glucose serves as an important source of energy for photoreceptors, like other neurons in the central nervous system. Similar to metabolically demanding tumors, the retina

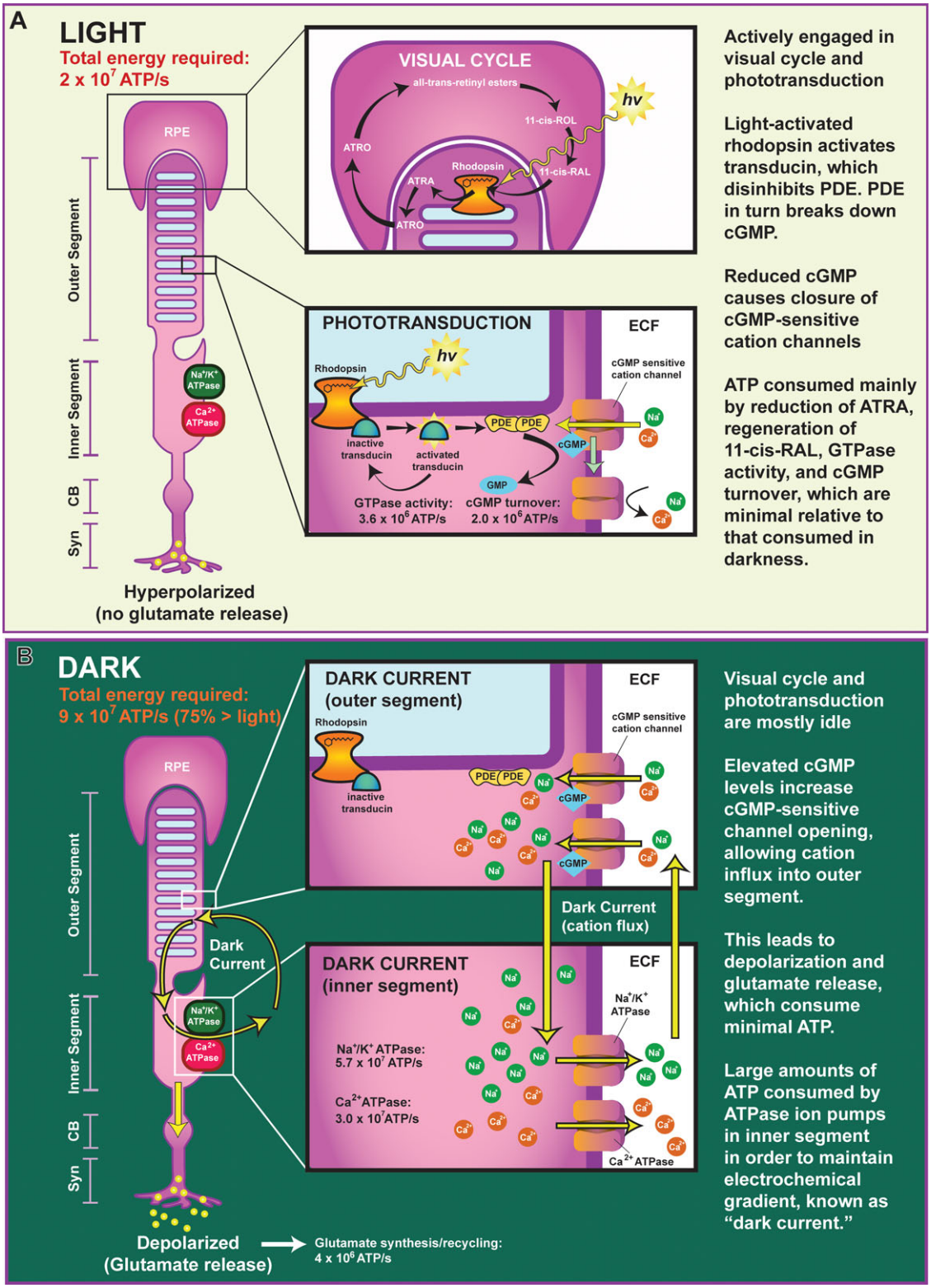

Fig. 2. Energy requirements of photoreceptors in light and dark. Schematic of photoreceptor and adjacent retinal pigment epithelium engaged in activities corresponding to light **(A)** and dark **(B)** conditions. The main ATP-consuming processes for each condition are outlined. ATP, adenosine triphosphate; GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate; s, second; RPE, retinal pigment epithelium; ECF, extracellular fluid; hv, light; 11-cis-RAL, 11-cis-retinal; 11-cis-ROL, 11-cis-retinol; ATRA, all-trans-retinal; ATRO, all-trans-retinol; Na^+, sodium ion; Ca^{2+}, calcium ion; PDE, phosphodiesterase; CB, cell body; Syn, synaptic terminal.

metabolizes the majority of its glucose into lactate via aerobic glycolysis rather than through oxidative phosphorylation for ATP production (Warburg et al., 1927; Joyal et al., 2018). Yet in pig retinal explants, only 20% of glucose is oxidized, suggesting that the retina uses alternate fuels for oxidative phosphorylation (Wang et al., 1997). Recently, palmitate was shown to be a possible fuel

substrate for mitochondrial energy production in photoreceptors (Joyal et al., 2016). Furthermore, mechanisms accounting for fuel partitioning in photoreceptors are poorly understood. Weak coupling of ATP synthesis and respiration in photoreceptors compared to other tissues suggests that photoreceptor energy production does not respond directly to energy demand but rather may fall under the control of photoreceptor signaling. Phototransduction, for instance, elevates citrate production by simultaneously increasing flux through citrate and decreasing flux through citric acid cycle intermediates (Du et al., 2016). This may occur via phototransduction-mediated depletion of calcium surrounding mitochondria clustered at the junction of outer and inner segments, thereby impacting the distribution of metabolites between matrix and cytosol (Krizaj & Copenhagen, 2002; Glancy & Balaban, 2012; Llorente-Folch et al., 2015). The increased citrate can then serve as a substrate to produce nicotinamide adenine dinucleotide phosphate (NADPH) required for the reduction and detoxification of all-trans-RAL generated by light-induced bleaching of rhodopsin (Adler et al., 2014). Among other metabolic changes, phototransduction also decreases metabolic flux through glycolysis and influences concentrations of 5′-GMP, ribose-5-phosphate, ketone bodies, and purines. Significantly, transcriptional coupling mechanisms also ensure precise matching of energy production with energy consumption (Du et al., 2016).

Oxygen consumption by photoreceptors

In order to produce ATP, photoreceptors require a blood supply of glucose and oxygen. Given the avascularity of the photoreceptor layers, photoreceptors normally function at low partial oxygen pressure (pO_2). Light-adapted photoreceptors primarily extract oxygen from the choroidal circulation, allowing their local pO_2 to reach up to 40 mmHg. However, the remarkably intense activity and oxygen consumption of photoreceptors in darkness reduce their surrounding minimum pO_2 tension to zero, triggering supplemental oxygen to be supplied from the central retinal artery and subsequently resulting in extension of a low oxygen tension environment to the inner retina (Linsenmeier, 1986; Braun et al., 1995). Such a model raises the concern that in disease states with suboptimal oxygen supply, pathologic hypoxia may develop in the inner portions of the retina when rod photoreceptors operate at maximum activity in darkness. To test whether such a concern is valid, longitudinal blood flow and oxygen measurements obtained with visible-optical coherence tomography were used to assess inner retinal oxygen metabolism ($IRMO_2$) in murine models of early type 1 diabetes. Notably, these analyses revealed alterations in $IRMO_2$ prior to detectable retinal vascular complications in early diabetes (Liu et al., 2017). Although the mechanism underlying changes in $IRMO_2$ are obscure, these findings suggest that abnormalities in oxygen metabolism precede and may contribute to DR. In line with this notion, oxygen supplementation in patients with diabetes reverses visual deficits typical of diabetic neuroretinopathy, measured using psychophysical and electrophysiological tests. Therefore, both preclinical and clinical evidence highly suggest that insufficient oxygen supply to the neural retina is an important contributor to vision loss in diabetes (Harris et al., 1996; Dean et al., 1997; Drasdo et al., 2002).

Manipulating light exposure as a therapy for DR

Although the evidence for retinal hypoxia in DR is strong, the reasons for its development are not so clear. One widely accepted mechanism involves hyperglycemia-induced damage of retinal microvasculature in early diabetes (Arden et al., 2005). To counteract such losses in oxygen supply due to microvessel compromise in diabetes, Dr. Arden proposed that reducing retinal oxygen demand could ease the strain on its vascular network. Since retinal ATP and oxygen use is higher in the dark than in light due to the dark current, as detailed above, he expected that retinal hypoxic stress in diabetes would be intensified by dark adaptation. Conversely, lowering rod photoreceptor dark current—by pharmacologic means or by use of continuous low-level illumination—would prevent DR. These predictions culminated in Arden's hypothesis that having people with diabetes sleep in light levels of 1–10 cd/m^2 would allow sufficient light to pass through the eyelids to prevent full dark adaptation, thereby providing beneficial effects for DR (Arden et al., 2005).

Clinical evidence for light therapy in DR

Arden's hypothesis was tested in several clinical trials that evaluated the efficacy of nighttime low-level light exposure on reduction of DR. Two short-term, early-phase prospective clinical trials showed improvement in DR measures in patients who wore custom-designed light masks while sleeping (Arden et al., 2010, 2011). However, in the follow-up phase 3 trial (CLEOPATRA), these light masks failed to show efficacy in DR, using reduction in preexisting noncentral diabetic macular edema as the primary end point (Sivaprasad et al., 2018). Likewise, in a STZ-rodent study, constant light exposure did not alleviate neuroretinal abnormalities during early stages of diabetes (Kur et al., 2016). Moreover, the CLEOPATRA trial noted challenges with wearing of the light masks during sleep, concluding this may not be a sustainable or effective option (Sivaprasad et al., 2018). Nevertheless, other clinical trials of light masks to prevent dark adaptation in DR are ongoing, including the Lahey Light II trial (LCID Study Number 2015-020), which is investigating a modified 520 µm LED light mask to prevent dark adaptation in refractory diabetic macular edema (Okada & Chhablani, 2018). Collectively, given the conflicting evidence from preclinical and clinical studies, the roles that light and darkness have on DR remain controversial.

Effects of light manipulation in experimental DR

Considering these negative findings, our group recently investigated the prediction that light deprivation, or prolonged dark adaptation, will worsen DR due to increased energy consumption in darkness. We shielded diabetic mice from light exposure and measured retinopathy outcomes. Unexpectedly, we found that light deprivation prevented the development of diabetes-induced delays in visual responses assessed by full-field electroretinography in both tested models of experimental diabetes: *db/db* mice, a type 2 diabetes model, and in STZ-treated mice, a type 1 diabetes model. Similarly, two mouse mutants with impaired rod responses to photostimulation—*Gnat1*$^{-/-}$ and *Rpe65*$^{-/-}$—were also protected from DR (Thebeau et al., 2020). These findings were consistent with an independent study in STZ-treated *Gnat1*$^{-/-}$ mice, demonstrating that they showed no increase in capillary atrophy, leukostasis, or inner retinal barrier loss in diabetes, compared to nondiabetic counterparts. Notably, the authors of that study observed persistent albumin leakage into the outer retina in diabetic *Gnat1*$^{-/-}$ mice (Liu et al., 2019). Collectively, these observations in light-deprived animals and models of *Gnat1* loss and *Rpe65* loss suggest that *light*-induced signaling from photoreceptors is

required for the pathogenesis of DR. Yet our results introduce an apparent mechanistic paradox, since light-deprived retinas require more energy than light-exposed retinas and add another level of complexity to the issue of light exposure and its effects on DR.

Mechanistic implications

The protective effects of light deprivation and phototransduction ablation clearly diverge from Arden's prediction that continuous light exposure would ameliorate DR by reducing the metabolic demands of dark adaptation. His prediction originated from a widely held view that diabetes leads to a primary vasculopathy, resulting in insufficient energy supply to the neural retina. An alternative view, in which diabetes causes a *primary neuropathy* of the retina with subsequent effects on the retinal vasculature (Honasoge et al., 2019; Thebeau et al., 2020), permits exploration of novel mechanisms by which light deprivation could inhibit the development of DR, which we will now discuss.

First, how does light-dependent signaling affect fuel substrate selection in photoreceptors experiencing chronic hyperglycemia during early diabetes? Notably, phototransduction itself drives metabolic flux in mouse retina, raising the possibility that it may also influence fuel substrate selection (Du et al., 2016). While aerobic glycolysis is a major metabolic pathway in photoreceptors, it is now becoming evident that the retina also uses fatty acid ß-oxidation for energy metabolism. As evidence, retinas from mice with deletion of the gene encoding very low-density lipoprotein receptor ($Vldlr^{-/-}$) show reduced retinal glucose uptake due to suppressed expression of the Glut1 transporter from high levels of circulating lipids. This suppression was mediated by the lipid sensor–free fatty acid receptor 1 (Ffar1), which is necessary for fatty acid uptake into photoreceptors. Moreover, disruption of Ffar1 caused abnormal retinal neovascularization, suggesting this metabolic pathway could be relevant to neovascular retinal disease, such as DR (Joyal et al., 2016). The presence of this interplay between glucose and lipid metabolism suggests that the retina is capable of adapting its fuel utilization to improve metabolic efficiency and match nutrient availability. This strategy is also employed by other metabolically demanding tissues in response to environmental stress in order to confer survival advantages (Joyal et al., 2018).

Clinical evidence implicating fatty acid ß-oxidation in DR has emerged from two large scale clinical trials (FIELD and ACCORD Eye studies), which found that fenofibrate-induced activation of PPARα, a nuclear receptor that modulates lipoprotein lipase expression and triglyceride metabolism, reduced the progression of DR by 30–40% (Keech et al., 2007; Group et al., 2010). This was supported by rodent models of pathological retinal angiogenesis, in which fenofibrate reduced retinal neovascularization and retinal vascular leakage (Chen et al., 2013). Although the precise mechanisms accounting for the beneficial effects of fenofibrate in the diabetic retina remain unclear, the drug could promote a fuel switch to fatty acids in photoreceptors to correct diabetes-associated retinal energy deficit and subsequent neural dysfunction. This understudied concept will require the further exploration of other control mechanisms for fuel substrate selection and the interplay between glucose and lipid metabolism in photoreceptor cells.

Beyond their newly identified roles in retinal metabolism, lipids also serve as the fundamental unit of photosensitive discs in photoreceptor outer segment. As these lipids are vulnerable to damage from light and oxidation, decreasing light exposure may reduce levels of oxidative damage to lipids in the outer segment of photoreceptors (Fu et al., 2020). The detrimental effects of light-induced lipid damage were elucidated in a study exposing rodents to constant light. In this study, light-induced oxidative stress reduced retinal levels of a critical fatty acid in the adult human retina, docosahexaenoic acid. Consequently, this led to changes in cellular membrane composition and rigidity, a phenomenon known to affect cellular metabolism in other cell types (Habib et al., 1987; Benedetto & Contin, 2019). Further studies on light-induced oxidative damage to photoreceptor lipids may provide novel insights into the role of retinal oxidative stress in DR.

In addition to modulating fuel substrate selection, increased energy demands confronting diabetic photoreceptors in dark-adapted conditions could potentially confer advantageous adaptations in metabolism. For instance, increased demand for ATP in darkness may give rise to mitochondrial expansion and increased oxidative capacity, resembling the effects seen after exercise on skeletal muscle (Meinild Lundby et al., 2018). Moreover, dark exposure could have effects on improving retinal insulin sensitivity, which is reduced in diabetes (Reiter et al., 2006).

Conceivably, dysfunctional photoreceptors may also participate in the non-cell autonomous progression of DR by disturbing the other retinal cell types with which they directly or indirectly communicate. Müller cells—the principal glia of the retina—respond to the metabolic demands of retinal neurons by regulating blood flow through their dependent vessels, a process referred to as neurovascular coupling or functional hyperemia (Metea & Newman, 2006). Specifically, in response to neural stimuli, Müller cells release vasoactive substances, such as nitric oxide (Newman, 2013). Although light stimulates vasodilation in healthy retina, patients with diabetes show decreased vascular reactivity to light (Berkowitz et al., 2015). Subsequent rodent studies attributed the reduced vascular reactivity in diabetes, in part, to elevated local nitric oxide concentration caused by a diabetes-induced increase in Müller cell expression of inducible nitric oxide. Restoration of neurovascular coupling in diabetic rodents occurred following treatment with aminoguanidine, an inhibitor of inducible nitric oxide (Mishra & Newman, 2010, 2011). As diabetic patients show signs of compromised neurovascular coupling as evidenced by reduced light-induced vascular reactivity, perhaps, a reduction in light-induced photoreceptor signaling could reduce stress on this system.

In addition to neuroglial interactions, photoreceptors may also communicate with leukocytes through release of proinflammatory cytokines in diabetes (Du et al., 2013; Kern, 2017). Interestingly, loss of phototransduction signaling in *Gnat1−/−* mice is associated with reduced diabetes-induced release of proinflammatory proteins, leukostasis, and leukocyte-mediated cytotoxicity to endothelial cells (Liu et al., 2019). This suggests that phototransduction, a process unique to photoreceptors, plays a significant role in the development of an inflammatory state and potentially, the eventual development of DR.

Visual cycle inhibition in DR

Clear limitations to strategies involving light modulation in the treatment of DR include the poor feasibility of keeping patients in prolonged low-light conditions and unintended effects on circadian cycles. Therefore, pharmacologic visual cycle inhibition represents another possible intervention for DR that targets photoreceptor metabolic physiology. In STZ-treated mice, systemic treatment with retinylamine—a retinoid that inhibits the critical visual cycle enzyme RPE65—significantly reduces diabetes-induced

oxidative stress, induction of inflammatory proteins, capillary permeability, and capillary degeneration (Liu et al., 2015). Slowing of the visual cycle could improve DR by several mechanisms. First, slowed visual cycle activity reduces 11-*cis* and all-*trans*-RAL levels in photoreceptor outer segments, thus reducing formation of precursors of bisretinoid toxins, such as A2E. Likewise, low all-*trans*-RAL levels also reduce the frequency of lipid peroxidation and generation of ROS. Second, light-dependent photoreceptor signaling could be permissive for DR development, as suggested by our own experience with *Gnat1*$^{-/-}$ and *Rpe65*$^{-/-}$ mice (Thebeau et al., 2020). Therefore, visual cycle inhibition might reduce these permissive signals. Third, despite an absence of light-dependent photoreceptor signaling, constitutive activity by visual chromophore-free opsin (apo-opsin) also prevents full dark adaptation, thereby reducing the metabolic demand as shown using manganese-assisted magnetic resonance imaging and oxygen tension measurements (Kubota et al., 2019). The relative contributions of decreased visual chromophore-derived toxin production, light-dependent photoreceptor signaling, and metabolic demands via apo-opsin signaling remain to be determined.

The promising results of visual cycle inhibition in diabetic animal models led to clinical testing of emixustat, the first clinical candidate in a class of nonretinoid, small-molecule compounds that reversibly inhibits the same target as retinylamine, RPE65. Early-phase clinical trials suggested that emixustat may reduce progression of DR in animal models. Currently, phase 3 clinical trials testing its efficacy in the treatment of DR, albeit in later stages of the disease, are ongoing (Kubota et al., 2019). As emixustat inhibits the visual cycle, potential adverse consequences on vision by emixustat treatment should be considered. Nyctalopia and dyschromatopsia were reported in emixustat clinical trials, consistent with emixustat's mechanism of action (Kubota et al., 2014, 2019). While emixustat shares the ability of retinylamine to inhibit RPE65, it also has the capacity to complex with and sequester all-trans-RAL via a condensation reaction. This may effectively reduce toxicity associated with excess free all-trans-RAL and raises the possibility that emixustat might operate in multiple ways to correct DR (Kubota et al., 2020).

Conclusion

DR is an important contributor to vision loss worldwide, and its prevalence is increasing. The clinical manifestations of the disease have historically been measured by observing characteristic vascular lesions. However, evidence from many groups in both clinical and preclinical settings strongly suggests that many retinal cell types are affected by diabetes, and perhaps, the initial insult could be a retinal neuropathy. Moreover, photoreceptors are clearly significant contributors to early retinal disease in diabetes. Stemming from observations of photoreceptor dysfunction in diabetes, modulation of phototransduction and inhibition of the visual cycle have emerged as novel modes of therapy for DR. However, the nature of putative contributions that the photoreceptor response to light may have on DR pathogenesis and therapy remains highly controversial and is an area of active and exciting investigation. Diabetes may impact several aspects of photoreceptor physiology in understudied ways, including fuel substrate selection, lipid oxidation, mitochondrial adaptation, and neurovascular coupling. It may also impact very well-characterized states of retinal pathophysiology, such as inflammatory responses, hypoxic drive, and oxidative stress. Moving forward, implementation of conditional genetic knockout or viral delivery strategies may elucidate how specific molecular perturbations within photoreceptors could impact any or all of these disease processes. In addition, temporal molecular profiling of photoreceptors over the extent of diabetes may reveal molecular abnormalities driving or marking the development and progression of DR. Ultimately, insights into photoreceptor physiology in DR may yield novel therapies that modulate the photoreceptor response to light to ameliorate diabetic retinal disease while maintaining intact vision.

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